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TITLE: Tuft Cell Regulation of miRNAs in Pancreatic Cancer

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14. ABSTRACT

To better understand the role of tuft cells in the pancreas and to define their effects on the pancreatic body as well as the initiation of pancreatic cancer. Tuft cells are present in the hollow organs of the digestive and respiratory tracts. They are characterized by long and blunt microvilli with prominent rootlets and by a well-developed tubulovesicular system in the supranuclear cytoplasm. Recent reports suggest that tuft cells may act as mechanoreceptors and are involved in chemosensensing of the microenvironment.

With the successful deletion of Dclk1 throughout the pancreatic ducts, we can begin characterization, which extends our understanding of the role tuft cells play within ducts and their broader effects on the overall pancreatic microenvironment. With the initial phase ductal specific KRAS-mutation now finished, we will soon be able to examine the role of ductal cells play in the initiation of neoplastic changes (via PDX-1-Cre). Then once completed in year two, the *Pdx-1-Cre;Dclk1$^{flox/flox}$;KRAS$^{LSL-G12D}$*, which removes Dclk1 expression from tuft cells and possibly the tuft cells as a whole, will dramatically extend the knowledgebase for the role of tuft cells in pancreatic neoplasia. These finding could very well provide the basis for the development of novel chemotherapeutic drugs targeting these specialized cells.

15. SUBJECT TERMS
None provided.

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INTRODUCTION: To better understand the role of tuft cells in the pancreas and to define their effects on the pancreatic body as well as the initiation of pancreatic cancer. Tuft cells are present in the hollow organs of the digestive and respiratory tracts. They are characterized by long and blunt microvilli with prominent rootlets and by a well-developed tubulovesicular system in the supranuclear cytoplasm. Recent reports suggest that tuft cells may act as mechanoreceptors and are involved in chemosensensing of the microenvironment.

BODY:

Aim/Task 1: To characterize tuft cell expression in the mouse pancreas. **(1 – 12 months)**

Tasks 1a and b: The initial breeding for the generation of the proposed *p48^{cre};KRAS^{G12D}* mouse model proved problematic and the rate of genotypically desirably offspring was not consistent with expected and proposed results. The animals numbers needed would not be reached at an appropriate time for the completed of proposed work. Therefore we sought to replace the current p48^{cre} breeding stock. Around and about 5/2013 email contact with Jackson Labs revealed the following "*Re: Under Development - Now Accepting Orders for Strain: B6.129S6(Cg)-Ptf1a$^{tm2(cre/ESR1)Cvw}$/J (Stock# 019378). This strain has arrived on our campus and is in the rederivation process. We encourage you to reserve your mice in advance by placing your order as soon as possible. The rederivation process takes approximately 6-7 months*". Currently we are expecting shipping distribution to be available on 11/18/2013 and this task to be completed in second year (12-24 month). During this time we also pursued as an alternative strategy the creation of *Pdx-1-Cre;KRAS^{G12D}* mice. This mouse model has just recently been successfully completed and we are now scaling-up breeding to experimental numbers. This will provide additional support for this aim in the second year (12-24 month).

Aim/Task 2: To determine the role of Dclk1 (DCAMKL-1) in pancreatic tuft cell regulation and regulation of miRNAs in tuft cells. (1 – 12 months)

Task 2a: Here we have successfully crossed *Pdx-1-Cre* to *Dclk1$^{flox/flox}$* (formally DCAMKL-1) and created the novel compound mouse model *Pdx-1-Cre;Dclk1$^{flox/flox}$*. This accomplishment came to fruition on or about 3/2013 with large-scale breeding beginning just after. Initial characterization experiments were conducted subsequently and confirmed that Dclk1 was in fact deleted from all pancreatic ductal regions as expected from utilizing the Pdx-1-Cre promoter (Figure 1). We have also characterized the expression of other tuft cell markers Cox1 and Cox2 (Figure 1). The full assessment of these mice for the proposed chronology is currently underway and will be completed early in year two.

Task 2b: Following the recent successful creation and preliminary characterization of the *Pdx-1-Cre;Dclk1$^{flox/flox}$* compound mice, completion of this task for these specific mice will occur early in year two. However initial mRNA and miRNA analysis utilizing RT-PCR has been completed for the control mice (WT) (Data not shown). Because of

the nature of microarray technology, this potion of this task will be performed when we have the full complement of mice from both Dclk1 deletion and control mice available at the applicable chronological time points.

Task 2c: The proposed miRNA profiling of Dclk1^{+} tuft cells from C57BL/6 wild type mice has been largely completed. Cells of the pancreata were isolated using Alexa Fluor conjugated Dclk1 antibody and FACS sorted into ve^{+} and ve^{-} subpopulations. These subpopulations were then subjected to RT-PCR analysis (Figure 2). Ultrastructural investigation of these cell subpopulations is currently underway and will be completed in year two.

Aim 3: To determine the mechanism by which DCAMKL-1+ tuft cells regulate pancreatic cancer initiation. (12 – 24 months)

For this aim are now crossing the completed *Pdx-1-Cre;Dclk1$^{flox/flox}$* with the KRAS$^{LSL-G12D}$ mouse. We are on schedule for this experimental timeline to be completed in year two.

KEY RESEARCH ACCOMPLISHMENTS:

- Successful creation of the novel compound mouse *Pdx-1-Cre;Dclk1$^{flox/flox}$*.
- Initial characterization of *Pdx-1-Cre;Dclk1$^{flox/flox}$* was completed which demonstrated the loss of Dclk1 expression within tuft cells in all pancreatic ducts.
- Successful creation of the novel compound mouse *Pdx-1-Cre;KRAS$^{LSL-G12D}$*

REPORTABLE OUTCOMES:

- Created the novel compound mouse *Pdx-1-Cre;Dclk1$^{flox/flox}$*
- Created the novel compound mouse *Pdx-1-Cre;KRAS$^{LSL-G12D}$*

CONCLUSION:

With the successful deletion of Dclk1 throughout the pancreatic ducts, we can begin characterization, which extends our understanding of the role tuft cells play within ducts and their broader effects on the overall pancreatic microenvironment. With the initial phase ductal specific KRAS-mutation now finished, we will soon be able to examine the role of ductal cells play in the initiation of neoplastic changes (via PDX-1-Cre). Then once completed in year two, the *Pdx-1-Cre;Dclk1$^{flox/flox}$;KRAS$^{LSL-G12D}$*, which removes Dclk1 expression from tuft cells and possibly the tuft cells as a whole, will dramatically extend the knowledgebase for the role of tuft cells in pancreatic neoplasia. These finding could very well provide the basis for the development of novel chemotherapeutic drugs targeting these specialized cells.

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May, R., Sureban, S.M., Lightfoot, S.A., Brackett, D.J., Postier, R.G., Ramanujam, R.P., Wyche, J.H., Han, Z., Rao, C.V., Anant, S. and Houchen, C.W. Identification of a novel putative pancreatic stem cell marker DCAMKL-1 in normal mouse pancreas. *American Journal of Physiology-Gastrointestinal and Liver Physiology*. **2010** Aug;299(2):G303-310.

Sureban, S.M., May, R., Lightfoot, S.A., Hoskins, A.B., Brackett, D.J., Postier, R.G., Ramanujam, R., Mohammed, A., Rao, C.V., Wyche, J.H., Anant, S. and Houchen, C.W. DCAMKL-1 regulates human pancreatic cancer epithelial-mesenchymal transition via a *miR-200a* microRNA-dependent mechanism. *Cancer Research*. **2011** Mar;15;71(6):2328-2338.

Sureban, S.M., May, R., Qu, D., Weygant, N., Chandrakesan, P., Ali, N., Lightfoot, S.A., Pantazis, P., Brackett, D.J., Rao, C.V., Postier, R.G. and Houchen, C.W. DCLK1 regulates pluripotency and angiogenesis via microRNA dependent mechanisms in pancreatic cancer. *PLoS One*. **2013** Sep;8(9):e73940.

APPENDICES: None

SUPPORTING DATA:

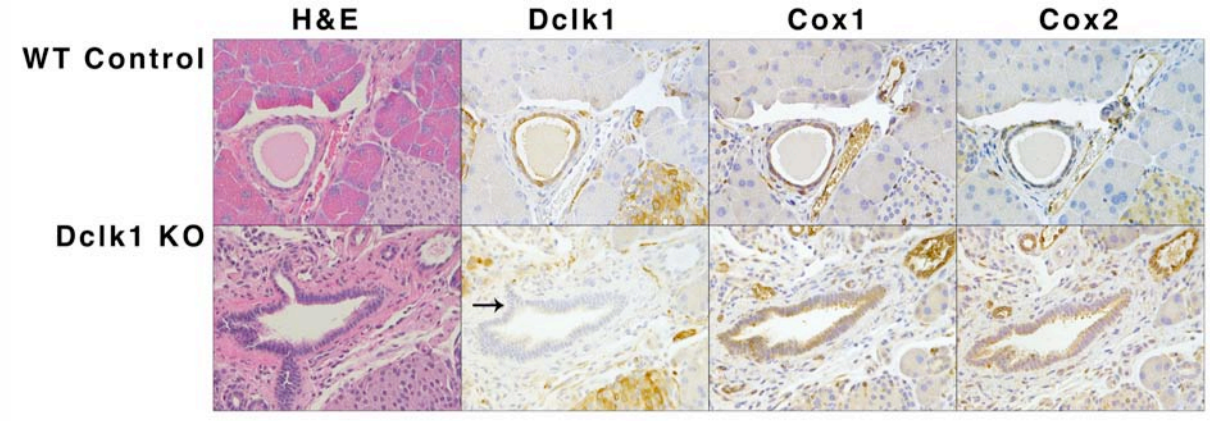

Figure 1: The novel compound mouse model *Pdx-1-Cre-;Dclk1$^{flox/flox}$* demonstrates the ductal-specific deletion of Dclk1. Top panel demonstrates the presence of pancreatic duct (H&E) with the expression of Dclk1, Cox1 and Cox2 seen in *Pdx-1-Cre* control mice (WT). The lower panel also demonstrates a pancreatic duct (H&E), but the notable absence of Dclk1 can be seen (arrow), the continued expression of tuft cell markers Cox1 and Cox2 can also been identified.

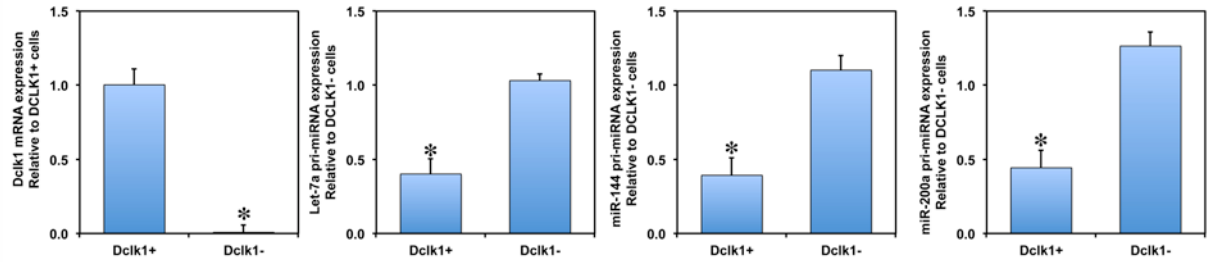

Figure 2: Expression of tumor suppressor miRNAs is low in Dclk1^{+} pancreatic tuft cells. Pancreatic cells from wild-type C57BL/6 mice were isolated based on the procedure published earlier (May *et al.,* 2010). Total cells were subjected to Dclk1-based fluorescent activated cell sorting protocols to isolate Dclk1^{+} and Dclk1^{-} cells. Total RNA was isolated from these cells and subjected to Real-time RTPCR analysis for Dclk1 mRNA, and other pri-miRNAs – *Let-7a, miR-144, miR-200a*. We observed higher levels of Dclk1 mRNA in Dclk1^{+} cells compared to Dclk1^{-} cells. Furthermore, we also observed significantly ($p<0.01$) lower expression of *Let-7a, miR-144* and *miR-200a*. These results are consistent with our previously published reports indicating that DCLK1 regulates these tumor suppressor miRNAs in human pancreatic cancer cell lines (Sureban *et al.,* 2011 and Sureban *et al.,* 2013).